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(54) Title: METHOD OF DECREASING CUTANEOUS SENESENCE (57) Abstract Epidermal cell, and thereby cutaneous senescence in a human is decreased by topically administering to human skin an ef- fective amount of a protein growth factor optionally in a pharmaceutically or cosmetically acceptable carrier.		

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5 METHOD OF DECREASING CUTANEOUS SENESCENCE

Technical Field

 The invention relates to a method of decreasing cutaneous senescence and thereby the stigmata of aging.

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Background and Related Art

 It is well known that as patients age the rate of epidermal cell replication and desquamation, i.e. turnover of cells, decreases or the epidermis becomes
15 senescent, this frequently produces a dull, aged appearance. In addition, the vascularity of skin decreases with time and the underlying collagenous framework undergoes structural fragmentation secondary to aging and photo-damage, hence elastosis; as a result,
20 wrinkles and sagging occur.

 "Senescence" at the cellular level results from inadequate DNA repair leading to disordered and/or nonexistent cell replication. Loss of mitotic control factors in the nucleus and cytoplasm including disordered
25 nuclear cytoplasmic exchange and permanent closing of microcirculatory capillary beds results in focal cell dropout and loss of cell and organelle membrane function.

 The lifetime effects of the damage include wrinkling and hardening of the skin with age. The skin
30 is made up of supportive material, including elastin and collagen. Collagen is a major protein component of the white fibers of connective tissue, such as cartilage and bone. White elastin is the major protein in the connective tissue of large blood vessels in the skin
35 which enables these tissues to stretch and resume their

original confirmation. Both collagen and elastin contain fibers that are linked together with imide bonds. It is believed that mammalian or human aging involves the oxidation of imide bonds to amide bonds with decreased elastic and flexible properties. A free radical mechanism is involved in wrinkling of the skin and results from the negative effects oxidation products which causes tissue aging.

U.S. patent 4,695,590 describes a method for retarding aging by administering synthetic chemicals, such as certain hydroxy diphenyl alkyl derivatives, preferably by oral administration. It would be desirable to avoid the internal administration of synthetic chemicals both for convenience and to avoid possible side effects of internally administered synthetic chemicals.

A variety of protein factors are known to be essential to the growth and differentiation of cells including epidermal cells. Many of these proteins extracted from tissues have been identified: such as epidermal growth factor (EGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and the like. U.S. patent 4,959,353 describes the use of epidermal growth factor for treating corneal wounds and U.S. patent 5,130,298 describes compositions of epidermal growth factor stabilized against degradation with metal cations and used for treating wounds. However, as these patents illustrate, protein growth factors have not been previously shown to decrease epidermal cell senescence in unabraded or nonwounded skin. It had been previously thought that large proteins such as growth factors could not penetrate uninjured or intact skin in order to reach the appropriate basal cell layers to increase cellular replication and thereby decrease epidermal cell senescence.

It would be desirable to have a simple method to decrease epidermal cell and thereby cutaneous senescence in humans with or without aesthetic and reconstructive surgery.

5

Summary of the Invention

The invention is directed to a method for decreasing the stigmata of aging in humans by topically administering to human skin a protein growth factor, optionally in a topical pharmaceutically or cosmetically acceptable carrier, in an amount that effectively decreases cutaneous senescence in humans.

It previously has been doubted that such growth factor proteins could reach the appropriate basal cell layer to produce increased cellular mitosis and hence replication. By contrast, this method of the invention results in one or more affects such as decreased senescence of epidermal cells thereby increasing the rate of cellular replication and desquamation producing a more youthful appearance; delaying cutaneous atrophy and the thinning of epidermis and dermis.

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Detailed Description of the Drawings

Figure 1 is a graphic representation of the data shown in Table 1 of Example 1 of the Flow Cytometric Analysis on patient skin after 30 days of treatment with epidermal growth factor and the skin of the untreated control. The results were expressed as the percentage of total skin cells in the actively dividing stage (S-phase).

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Figure 2 is a photograph of postauricular human skin 30 days after application of cream vehicle only as the untreated control.

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Figure 3 is a photograph of postauricular human skin after 30 days of treatment with epidermal growth factor.

5 Figure 4 is a photograph of intact postauricular human skin after 30 days of application of cream vehicle alone as untreated control.

Figure 5 is a photograph of intact postauricular human skin 30 days after treatment with epidermal growth factor.

10

Detailed Description of the Invention

For convenience, the following definitions are provided for use in describing the invention.

15 Definitions

As used herein, "epidermal cells" means the outer epithelial portion of the skin, i.e. cuticle.

20 As used herein, "cutaneous" is synonymous with the skin.

As used herein, "skin" means the membrane covering of a human body. The layers of the skin are the epidermis and the dermis.

25 As used herein "epidermal cell or cutaneous senescence" means the state of growing old and particularly damage to the epidermal cells of human skin which results from partial damage or complete destruction of the cells, conversion of imide bonds to amide bonds in collagen and/or elastin caused by toxic byproducts of
30 oxygen metabolism, free-radical pathology mechanisms or by photo-damage and generalized aging.

Thus, decreasing epidermal cell and thereby cutaneous senescence in a human means reducing or inhibiting senescence, including one or more affects such
35 as reversing photo-damage or other regenerative effects,

such as increasing underlying skin vascularity, increasing the rate of cellular replication and desquamation producing a more youthful appearance, increasing collagen synthesis and homogeneity, delaying cutaneous atrophy and thinning of epidermis and dermis, and the like. While not being bound by any theory, it is also believed that the method of the invention results in decreased elastosis, increasing underlying vascularity, increasing collagen synthesis and structural homogeneity and reversing photo-damage.

As used herein, "effective amount to decrease senescence" means the amount of protein growth factor or composition thereof in a topical pharmaceutically or cosmetically acceptable carrier which is applied to human skin to decrease cutaneous senescence in a human.

As used herein "flow cytometric analysis" is a method of photon beam cellular detection which measures the percentage of the total skin cells in the actively dividing stage--called the S-phase. Flow cytometry can be used to establish whether a protein growth factor treatment of the invention increased the baseline cellular division rate of the treated skin.

As used herein "protein growth factor" includes both native and recombinant protein growth factors as well as biologically active fragments and analogs thereof capable of decreasing epidermal cell and thereby cutaneous senescence. These factors and fragments or analogs thereof are well known.

Any protein growth factor capable of decreasing senescence of epidermal cells and thereby decreasing cutaneous cell senescence in a human can be used including, but not limited to, at least one of the following: epidermal growth factor (EGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), macrophage-

derived growth factor (MDGF), tumor angiogenesis factor (TAF), endothelial cell growth factor (ECGF), hypothalamus-derived growth factor (HDGF), transforming growth factor-beta (TGF- β), transforming growth factor
5 alpha (TGF- α), retina-derived growth factor (RDGF), heparin-binding growth factor (HGF) and mixtures of two or more of these growth factors.

Preferred because of their availability and senescence decreasing properties are EGF, IGF, PDGF and
10 FGF and mixtures thereof, including (a) IGF and EGF, (b) EGF and PDGF, (c) EGF, PDGF and IGF, (d) IGF and PDGF, (e) EGF and FGF, (f) EGF, PDGF and FGF, (g) IGF and FGF, (h) FGF and PDGF, (i) PDGF, FGF and IGF, and
(j) PDGF, FGF, IGF and EGF.

15 Although it can be desirable and may even be required by regulatory agencies, to apply a human protein growth factor to humans, it is not a requirement of the method. Thus, a protein growth factor having a human source can be administered to humans but also a protein
20 growth factor having a non-human source, such as rat, bovine, canine and the like can be administered to humans.

Likewise, the tissue source of the protein growth factor is not critical and includes, but is not
25 limited to, brain, pituitary, hypothalamus, chondrosarcoma, cartilage, placenta and the like. Preferably the tissue source is human tissue.

A particular benefit of the invention is a simple method of topical administration to the skin of a
30 composition for decreasing epidermal cell senescence in a human which does not require the intact skin to have been pretreated to stimulate cell growth, particularly a simple method of topical administration to the skin not requiring abrading of the intact skin by a plastic
35 surgery technique or wounding in any way.

While the protein growth factor can be topically administered alone,, it can also be desirable to administer the protein growth factor in admixture with a topical pharmaceutically or cosmetically acceptable carrier.

By "topical pharmaceutically acceptable carrier" as used herein is meant any substantially non-toxic carrier conventionally usable for topical administration of pharmaceuticals in which the protein growth factor will remain stable and bioavailable when applied directly to the skin surface. For example, the protein growth factor can be dissolved in a liquid, dispersed or emulsified in a medium in a conventional manner to form a liquid preparation or is mixed with a semi-solid (gel) or solid carrier to form a paste, powder, ointment, cream, lotion or the like.

Suitable topical pharmaceutical acceptable carriers include water, petroleum jelly (vaseline), petrolatum, mineral oil, vegetable oil, animal oil, organic and inorganic waxes, such as microcrystalline, paraffin and ozocerite wax, natural polymers, such as xanthanes, gelatin, cellulose, collagen, starch, or gum arabic, synthetic polymers, such as discussed below, alcohols, polyols, and the like. Preferably, because of its non-toxic topical properties, the carrier is a water miscible carrier composition that is substantially miscible in water. Such water miscible topical pharmaceutical acceptable carrier composition can include those made with one or more appropriate ingredients set forth above but can also include sustained or delayed release carrier, including water containing, water dispersable or water soluble compositions, such as liposomes, microsponges, microspheres or microcapsules, aqueous base ointments, water-in-oil or oil-in-water emulsions, gels or the like.

In one embodiment of the invention, the topical pharmaceutically acceptable carrier comprises a sustained release or delayed release carrier. The carrier is any material capable of sustained or delayed release of the protein growth factor to provide a more efficient administration resulting in one or more of less frequent and/or decreased dosage of the protein growth factor, ease of handling, and extended or delayed effects on decreasing epidermal cell senescence. The carrier is capable of releasing the protein growth factor when exposed to any oily, fatty, waxy, or moist environment on the area being treated or by diffusing or by release dependent on the degree of loading of the factor to the carrier in order to obtain releases of the factor. Non-limiting examples of such carriers include liposomes, microsponges, microspheres, or microcapsules of natural and synthetic polymers and the like. Examples of suitable carriers for sustained or delayed release in a moist environment include gelatin, gum arabic, xanthane polymers; by degree of loading include lignin polymers and the like; by oily, fatty or waxy environment include thermoplastic or flexible thermoset resin or elastomer including thermoplastic resins such as polyvinyl halides, polyvinyl esters, polyvinylidene halides and halogenated polyolefins, elastomers such as brasiliensis, polydienes, and halogenated natural and synthetic rubbers, and flexible thermoset resins such as polyurethanes, epoxy resins and the like. Preferably, the sustained or delayed release carrier is a liposome, microsphere, microsponge, or gel.

The compositions used in the method of the invention are applied directly to the skin cell areas to be treated. While not required, it is desirable that the topical composition maintain the factor at the desired location for about 24 to 48 hours.

If desired, one or more additional ingredients conventionally found in topical pharmaceutical or cosmetic compositions can be included with the carrier: such as a moisturizer, humectants, odor modifier, buffer, pigment, preservative, vitamins such as A, C and E, emulsifier, dispersing agent, wetting agent, odor-modifying agent, gelling agents, stabilizer, propellant, antimicrobial agents, sunscreen, enzymes and the like. Those of skill in the art of topical pharmaceutical formulations can readily select the appropriate specific additional ingredients and amounts thereof. Suitable non-limiting examples of additional ingredients include super oxide dismutase, stearyl alcohol, isopropyl myristate, sorbitan monooleate, polyoxyethylene stearate, propylene glycol, water, alkali or alkaline earth lauryl sulfate, methylparaben, octyl dimethyl-p-amino benzoic acid (Padimate O), uric acid, reticulin, polymucosaccharides, hyaluronic acids, aloe vera, lecithin, polyoxyethylene sorbitan monooleate, Vitamin A or C, tocopherol (Vitamin E), alpha-hydroxy or alpha-keto acids such as pyruvic, lactic or glycolic acids, or any of the topical ingredients disclosed in U.S. patents 4,340,586, 4,695,590, 4,959,353 or 5,130,298, each incorporated herein by reference.

Because a more youthful and pleasing appearance is the generally desired result of the method of the invention, the topical carrier can also be a topical cosmetically acceptable carrier. By "topical cosmetically acceptable carrier" as used herein is meant any substantially non-toxic carrier conventionally usable for topical administration of cosmetics in which the protein growth factor will remain stable and bioavailable when applied directly to the skin surface. Suitable cosmetically acceptable carriers are known to those of skill in the art and include cosmetically acceptable

liquids, creams, oils, lotions, ointments, gels, or solids, such as conventional cosmetic night creams, foundation creams, suntan lotions, sunscreens, hand lotions, make-up and make-up bases, masks and the like.

5 Thus, to a substantial extent topical cosmetically acceptable carriers and pharmaceutically acceptable carriers are similar, if not often identical, in nature so that most of the earlier discussion on pharmaceutically acceptable carriers also applies to
10 cosmetically acceptable carriers. The compositions can contain other ingredients conventional in cosmetics including perfumes, estrogen, Vitamin A, C and E, alpha-hydroxy of alpha-keto acids such as pyruvic, lactic or glycolic acids, lanolin, vaseline, aloe vera, methyl or
15 propyl paraben, pigments and the like.

The effective amount of the protein growth factor or protein growth factor in the compositions used to decrease epidermal cell senescence in a human can vary depending on such factors as condition of the skin
20 (severity of senescence), age of the skin, the particular protein growth factor employed, the type of formulation and carrier ingredients used, frequency of administration, overall health of the individual being treated and the like. The precise amount for any
25 particular patient use can be determined by those of skill in the pharmaceutical art taking into consideration these factors and the present disclosure. By way of non-limiting example, when the protein growth factor is EGF, IGF, FGF or PDGF, the factor is usually administered to
30 humans at a daily dosage of from about 1 microgram per ml mg to about 0.1 microgram per ml mg, preferably about 1 microgram 1 ml mg. Preferably the factor is administered in at least two doses and no more than about six doses per day, or less when a sustained or delayed release form
35 is used.

The compositions for topical administration usually contain from about 0.0001% to about 90% by weight of the protein growth factor compared to the total weight of the composition, preferably from about 0.5% to about 20% by weight of the factor to composition, and especially from about 2% to about 5% by weight of factor to the composition.

The protein growth factor is administered by applying a coating or layer of the protein growth factor or composition thereof to the skin area desired to be treated. As a practical matter of convenience, the applied material is rubbed into the skin. Applications need not be rubbed into the skin and the layer or coating can be left on the skin overnight.

The above description of the methods and compositions of the invention are provided to illustrate the invention and should not be regarded as limiting it in any way. Variations by changing or modification or the substitution of equivalent materials will be apparent to those of skill in the art.

All patents and publications referred to herein are incorporated in their entirety by reference thereto in this specification.

25 Examples

The invention is illustrated by the following examples which should not be regarded as limiting the invention in any way.

30 Example 1

Nine pre-operative facelift surgery patients underwent treatment of postauricular skin with a topical water-miscible and cosmetically and pharmaceutically acceptable carrier cream vehicle alone on one side of the face and the same vehicle as a carrier for 0.1 micrograms

of human epidermal growth factor per milliliter of cream vehicle on the other side of the face. The skin test areas were approximately the size of a postage stamp and were included in areas to be excised in subsequent facelift surgery. These test areas were treated twice daily for 30 days prior to the surgery. After excision during surgery, the skin test specimens were submitted for flow cytometric analysis. Results of these analyses are set forth in Table 1 below.

10

Table 1. Flow Cytometric Analysis of Postauricular Skin After 30 Days Treatment with EGF

15	S-PHASE			
	<u>Patient No.</u>	<u>Age (years)</u>	<u>EGF</u>	<u>Vehicle Alone</u>
	1	62	18.7	2.2
	2	57	10.1	3.4
	3	41	6.0	6.1
20	4	47	14.9	3.8
	5	45	12.0	5.3
	6	67	13.7	2.9
	7	54	8.5	4.8
	8	59	9.6	4.9
25	9	65	12.8	3.7

Results set forth in Table 1 above demonstrated that there was a statistically significant difference between the EGF treated skin and the untreated control skin. EGF treated skin demonstrated an increased percentage of epidermal cells actively dividing (S-phase). Also, the increase was much more profound in older individuals, 45 years or older. In the experiments of this example, EGF reversed the senescence of epidermal

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cells by stimulating skin epidermal cell division in unwounded skin.

Figure 1 is a graphic representation of the same data set forth in Table 1 above.

5 Figure 2 provides a photograph of the skin of a histological slide of the right sided postauricular skin of patient 6 (age 67) after 30 days of application with a topical water-miscible cream vehicle without epidermal growth factor as untreated control. This untreated skin
10 exhibited absence of rete pegs, decreased epidermal thickness, poor collagen organization in the dermis and a fragmented stratum corneum. The cellular mitotic activity in the epidermis appeared limited. Elastosis was present in the dermis. All of these traits are
15 associated with the aging process.

 Figure 3 of the invention provides a photograph of a histological slide of the left sided postauricular skin (opposite side of Figure 2) of patient number 6 after 30 days of treatment with the EGF cream. This
20 treated skin exhibited normal epidermal and dermal architecture. There was a presence of the natural rete peg configuration, increased thickness of the stratified squamous epidermis, more mitotic activity in the basilar cell layers and an orderly and thickened stratum corneum
25 as compared to the untreated skin. In addition, the dermis showed orderly collagenous framework with decreased elastosis. These results are compatible with youthful skin and the reversal of the histological signs of aging.

30 Figure 4 is a photograph of untreated intact postauricular skin of patient 6. The untreated skin had a scaly, dull and irregular appearance produced by an irregular adherent stratum corneum. There was a lack of homogeneity with areas of pigmentation produced by
35

actinic and senile keratoses. Overall, there was an aged and dull appearance.

Figure 5 is a photograph of the intact postauricular skin of patient 6 30 days after treatment with the EGF cream. There was a much fresher and less irregular appearance to the treated skin as compared to untreated skin in Figure 4. The scaliness was drastically decreased as was the irregular pigmentation. The thickened adherent stratum corneum was absent.

10 The results reveal that the test areas treated with epidermal growth factor have a significantly thicker epidermis as well as underlying dermis. There is no evidence of neoplasia or metaplasia in the study sights. Immunofluorescence for DNA and RNA reveals there is a
15 much higher mitotic activity of epidermal cells treated with epidermal growth factor. The subjective grading of the test areas reveal that at eight weeks those test areas treated with the growth factor shows significantly greater skin turgor and decreased wrinkling as well as
20 increased homogeneity. From this study it is concluded that topical epidermal growth factor in a water-miscible carrier when applied to intact uninjured skin reverses the cutaneous senescence or aging process. The mechanism by which these results occur are due to mitotic
25 stimulation by the growth factor which results in increased epidermal cell replication and desquamation. No untoward side effects such as tumor development, rash, ulceration, or erythema from the growth factor are observed.

30

Example 2 (Not invention)

A prospective randomized double blinded clinical trial using skin graft donor sites to determine whether recombinant epidermal growth factor (EGF) would
35 accelerate the rate of epidermal cell regeneration in

humans was conducted as described in Brown, et al.,
"Enhancement of Wound Healing by Epidermal Growth Factor:
An Initial Clinical Report," New England Journal of
Medicine, 321:76-79 (1989), by creating paired donor
5 sites in 12 human patients who required skin grafting for
either burns or reconstructive surgery. One donor site
from each patient was treated topically with silver
sulfadiazine cream and one was treated with silver
sulfadiazine cream containing human epidermal growth
10 factor (10 micrograms per ml). The donor sites were
photographed daily and healing was measured with the use
of computerized planimetric analysis.

The donor sites treated with silver
sulfadiazine containing epidermal growth factor had an
15 accelerated rate of epidermal cell regeneration in all 12
patients as compared with the donor sites treated with
silver sulfadiazine alone. Treatment with epidermal
growth factor significantly decreased the average length
of time to 25% and 50% healing by approximately one day
20 and to 75% and 100% healing by approximately by 1.5 days
($P < 0.02$). Histological evaluation of punch biopsy
specimens taken from the centers of donor sites 3 days
after the onset of healing supported these results.

A planimetric analysis was done of the rate of
25 epidermal regeneration at paired donor sites of the 12
patients treated with either silver sulfadiazine cream
alone (squares) or silver sulfadiazine cream containing
(EGF) as the percentage of the original wound area that
was healed plotted against the number of days after
30 surgery.

While the time of onset of healing varied
somewhat from patient to patient, this study demonstrated
that the epidermal growth factor did accelerate healing
of partial thickness skin wounds but there was no
35 indication that the growth factor could be effective on

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uninjured skin or in decrease epidermal cell senescence of intact or uninjured skin.

Example 3 (Not invention)

5 Epidermal growth factor (EGF) was evaluated for its effect on topical treatment of healing chronic wounds in a prospective open label cross over trial as described in Brown, et al., "Stimulation of Healing of Chronic Wounds by Epidermal Growth Factor," Plastic &
10 Reconstructive Surgery, 88:189 (1991), in which five human males and 4 females who ranged in age from 40 to 72 years were enrolled. Four patients had adult onset diabetes mellitus, two had rheumatoid arthritis, two had old burn scars, and one had failed an abdominal incision.
15 The average duration of the ulcer prior to treatment with epidermal growth factor was 12 months (range 1-48 months). Following failure of the wound to heal with conventional therapies including debridement, skin grafts, and vascular reconstruction, the wounds were
20 treated twice daily with silvadene alone for periods ranging from 3 weeks to 6 months. No evidence of healing was observed in any patient's wounds during silvadene treatment alone and patients were crossed over to twice a day treatments with silvadene containing epidermal growth
25 factor (10 mg/ml). The wounds of 8 patients healed completely with epidermal growth factor silvadene treatment in an average of 34 days and did not recur for a period ranging from 1 to 4 years.

 This study demonstrated that the epidermal
30 growth factor did indeed enhance healing of chronic wounds but there was no indication that the growth factor could be effective on uninjured skin or decrease epidermal cell senescence of intact or uninjured skin.

What is claimed is:

1. A method for decreasing cutaneous cell senescence in a human which comprises topically administering to human skin a senescence decreasing effective amount of a protein growth factor optionally in a topical pharmaceutically or cosmetically acceptable carrier.

2. The method according to claim 1 wherein said growth factor is epidermal growth factor (EGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), macrophage-derived growth factor (MDGF), tumor angiogenesis factor (TAF), endothelial cell growth factor (ECGF), hypothalamus-derived growth factor (HDGF), retina-derived growth factor (RDGF), heparin-binding growth factor (HGF), transforming growth factor-beta ($\text{TGF-}\beta$), transforming growth factor alpha ($\text{TGF-}\alpha$), and mixtures of two or more of said growth factors.

3. The method according to claim 2 wherein said growth factor comprises epidermal growth factor (EGF).

4. The method according to claim 2 wherein said growth factor comprises insulin-like growth factor (IGF).

5. The method according to claim 2 wherein said growth factor comprises platelet-derived growth factor (PDGF).

6. The method according to claim 2 wherein said growth factor comprises fibroblast growth factor (FGF).

5 7. The method according to claim 2 wherein
said growth factor comprises (a) IGF and EGF, (b) EGF and
PDGF, (c) EGF, PDGF and IGF, (d) IGF and PDGF, (e) EGF
and FGF, (f) EGF, PDGF and FGF, (g) IGF and FGF, (h) FGF
and PDGF, (i) PDGF, FGF and IGF, or (j) PDGF, FGF, IGF
10 and EGF.

8. The method according to claim 1 wherein said skin comprise intact skin.

15 9. The method according to claim 1 wherein
said topical pharmaceutically acceptable carrier is a
water-miscible carrier.

 10. The method according to claim 9 wherein
20 said water-miscible carrier is water, petroleum jelly,
petrolatum, mineral oil, vegetable oil, animal oil, wax
or a polymer.

 11. The method according to claim 1 wherein
25 the carrier is a sustained or delayed release carrier.

 12. The method according to claim 11 wherein
the sustained or delayed release carrier is a liposome,
microsphere, microsphere, or gel.

30

 13. A method to reduce or delay cutaneous cell
atrophy in a human which comprises topically
administering to the human skin an effective amount of a
protein growth factor optionally in a pharmaceutically or
35 cosmetically acceptable carrier.

14. A method to reduce or delay thinning of the epidermis and dermis in human skin which comprises topically administering to the human skin an effective amount of a protein growth factor optionally in a topically pharmaceutically or cosmetically acceptable carrier.

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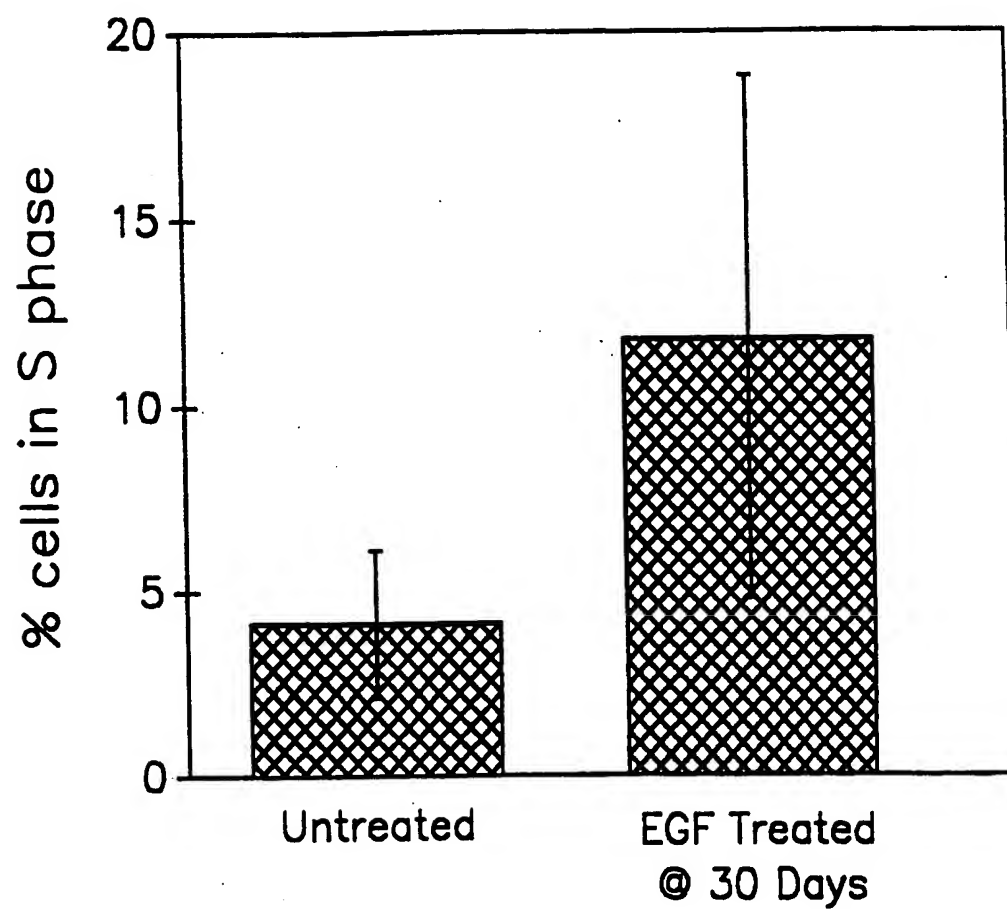


FIG. I

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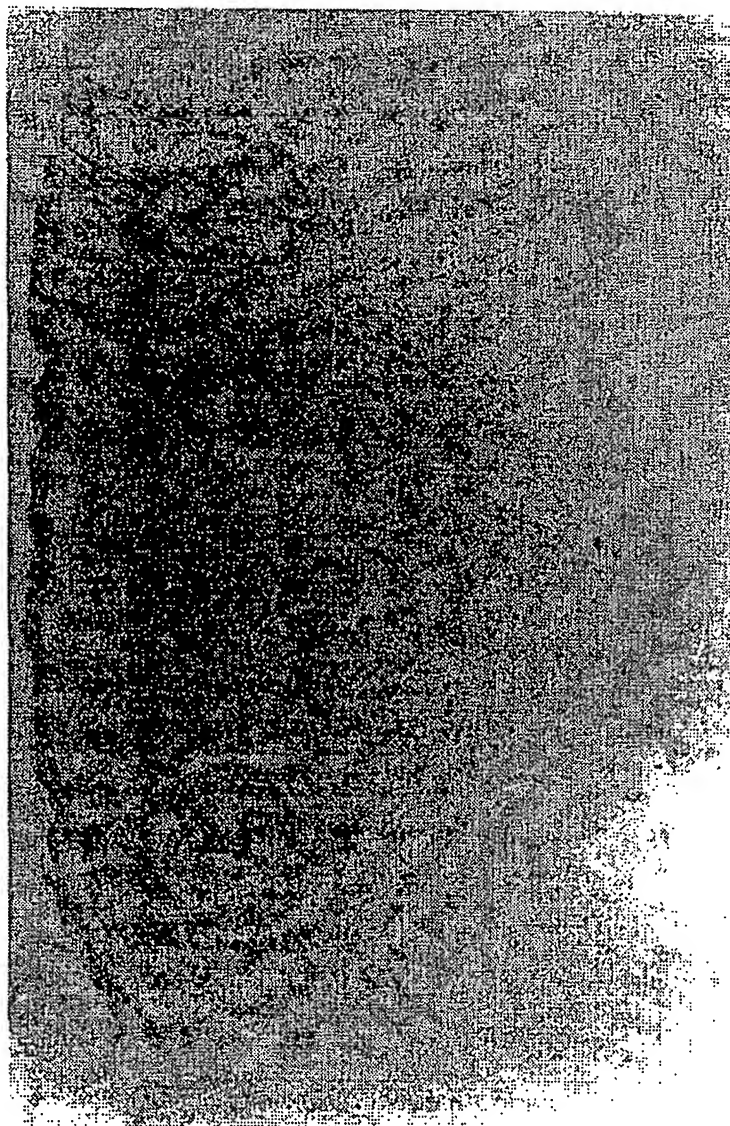


FIG. 2

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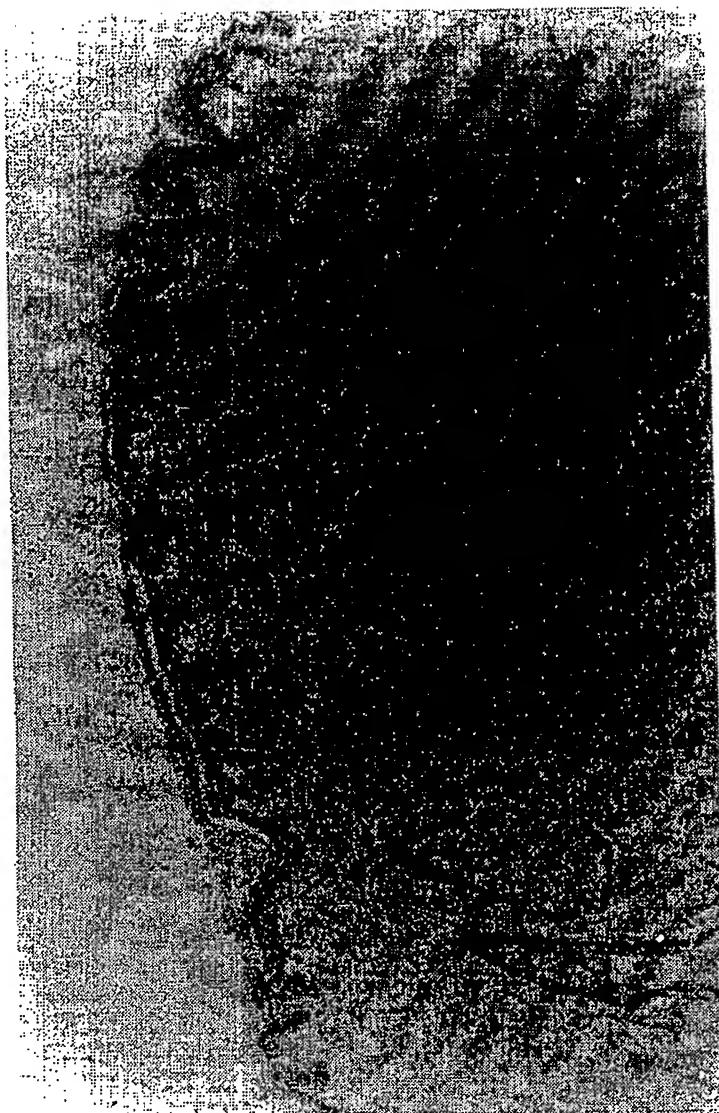


FIG. 3

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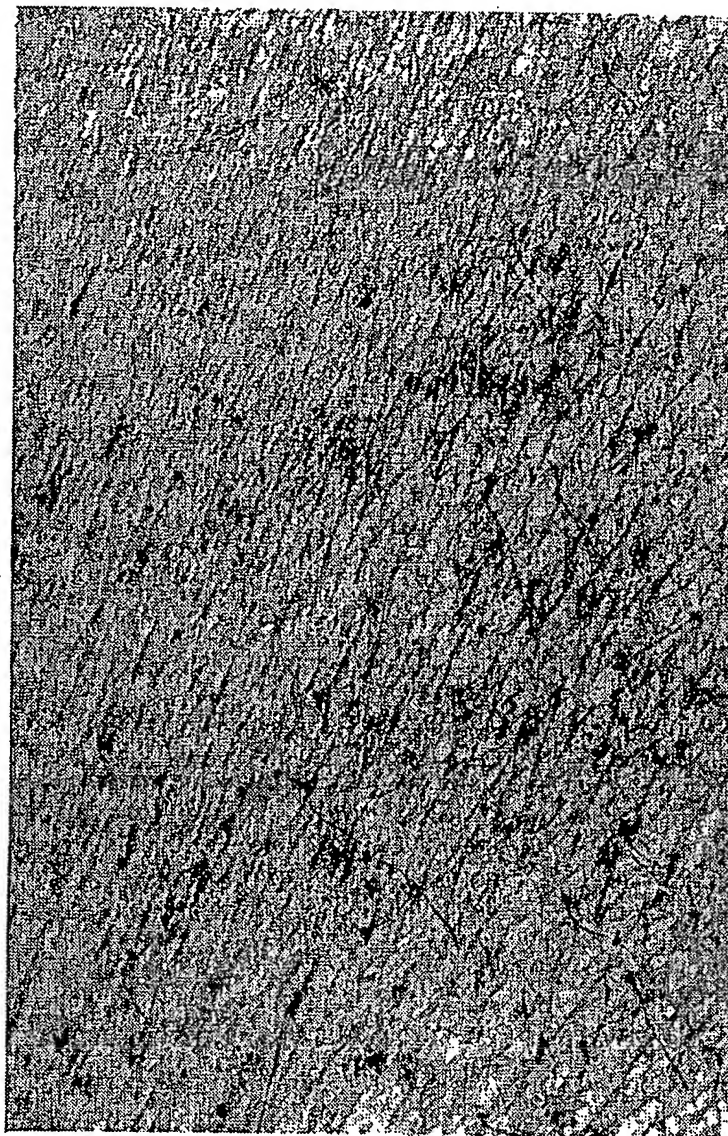


FIG. 4

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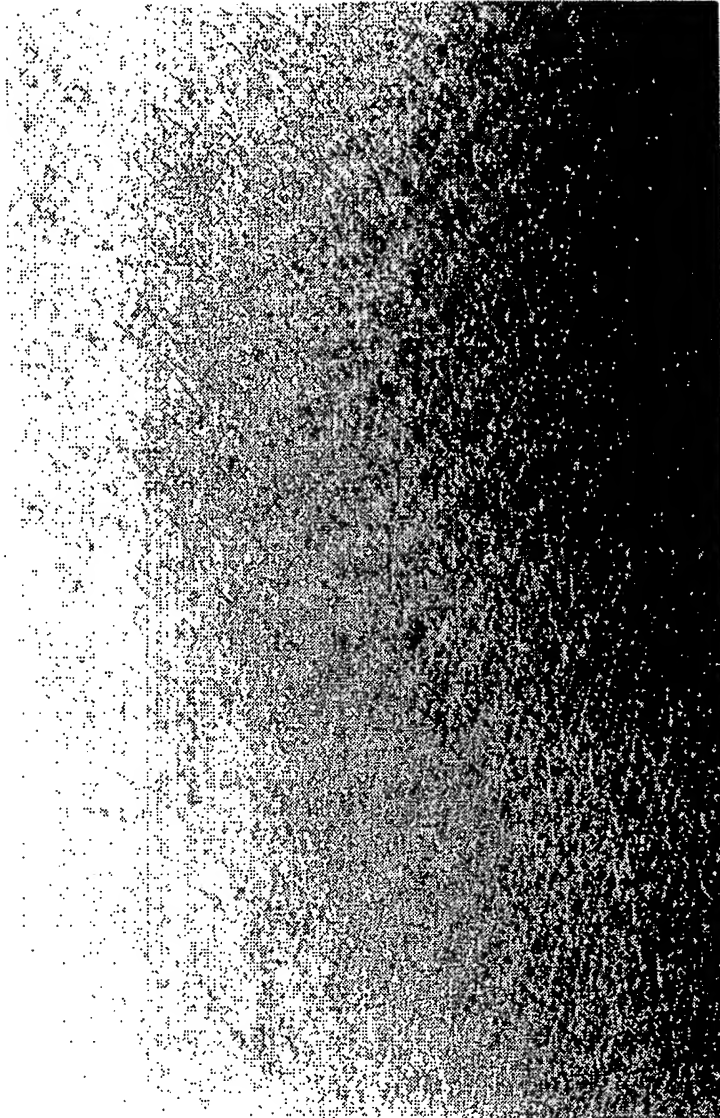


FIG. 5

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